Fluorinated Isatin Derivatives. Part 2. New *N*-Substituted 5-Pyrrolidinylsulfonyl Isatins as Potential Tools for Molecular Imaging of Caspases in Apoptosis

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Caspases are responsible for the execution of the cell death program and are potentially suitable targets for the specific imaging of apoptosis in vivo. A series of *N*-1-substituted analogues of the small molecule nonpeptide caspase inhibitor (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**), which may be useful for the development of caspase-targeted radioligands, were synthesized and their inhibition potencies were evaluated in vitro. Two of the most powerful techniques to introduce fluorine into organic compounds, viz, bromofluorination of olefins and fluorohydrin synthesis by ring-opening of epoxides, were used. Most of the target compounds are potent inhibitors of the two effector caspases-3 and -7. Furthermore, the ^{18}F-radiolabeled model compound (*S*)-1-[4-(1-[^{18}F]fluoro-2-hydroxyethyl)benzyl]-5-[1-(2-methoxymethyl-pyrrolidinyl)sulfonyl]isatin ([^{18}F]**37**), a putative tracer for the noninvasive imaging of apoptosis by positron emission tomography (PET) was synthesized by nucleophilic epoxide ring-opening of its precursor **36**. The radiochemistry utilized in the ^{18}F-fluorination reverted to carrier-added [^{18}F]$Et_3N \cdot 3HF$, a new fluorine-18 source for radiolabeling.

Introduction

Apoptosis is a natural form of cell death and is an energy dependent and genetically controlled process without inflammatory response that maintains homeostasis in proliferating tissues in adults.[1,2] Dysregulation of apoptosis is linked to increased cell proliferation or enhanced cell death, resulting in a variety of diseases. While an increased apoptosis rate occurs in acute myocardial infarction,[3] atherosclerosis,[4,5] allograft rejection,[6−9] stroke,[10−12] and neurodegenerative disorders;[13−15] decreased apoptosis rate can be observed during tumorigenesis,[16−19] autoimmune diseases,[20] and viral infections.[21] Both the diagnosis and therapy of such diseases demand noninvasive and serial imaging of apoptosis in vivo, e.g., as a surrogate marker of successful chemotherapy of tumors. Therefore, apoptosis imaging with the scintigraphic methods like single photon emission computed tomography (SPECT[a]) and positron emission tomography (PET) would be a clinically important tool for disease management and is still one of the greatest challenges in modern medicine. For this, a biological target for specific and exclusive apoptosis imaging is crucial.

The intracellular death enzyme class called caspases (*c*ysteinyl *asp*artate-specific prote*ases*) constitute such a specific target. During the apoptosis signaling cascade, caspases become activated, contributing to the disassembly of the apoptotic cell and hence execute the common final path of the programmed cell death process. Apoptosis involves two different classes of caspases, namely the initiator caspases (caspase-2, -8, -9, and -10), whose main function is the activation of the effector caspases, caspase-3, -6, and -7. In apoptosis, the physiological changes (e.g., cleavage of the DNA repair enzyme poly(ADP-ribose)polymerase-1, cytoskeleton proteins, and nuclear laminins) together with the morphological changes (nuclear membrane damage, membrane blebbing, and DNA strand breaks) are brought about by the effector caspases.[22,23] Caspases-1, -4, -5, and -11 constitute the third class but are not believed to play an active role in apoptosis.[24]

On the basis of the decisive role of caspases in apoptosis, the development of structurally diverse caspase inhibitors has been the focus of pharmaceutical drug development in recent years. These drugs are designed to therapeutically prevent apoptosis and rescue tissues.[25] Apart from therapeutic applications, these inhibitors could also be used for molecular imaging of caspase activity because they typically bind to activated caspases. However, direct in vivo application of labeled peptide-based caspase inhibitors as caspase imaging agents failed due to poor cell permeability.[26] Nonpeptidic 5-pyrrolidinylsulfonyl isatins have been shown to selectively inhibit the downstream caspases-3 and -7 in vitro.[24,27−32] These compounds do covalently and reversibly bind to the active site of the activated caspase enzymes. The C-3 carbonyl carbon of the isatin skeleton acting as an electrophile forms a thiohemiketal with the nucleophilic Cys thiolate function, and this in turn happens when the dicarbonyl functionality of the isatin binds to the Cys residue of the active site. It is for these reasons that radiolabeled 5-pyrrolidinylsulfonyl isatins were suggested as radiopharmaceuticals for clinical imaging of apoptosis by SPECT or PET.[32−35]

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) (Scheme 1)[28] was chosen as lead structure for the development of caspase inhibitors because the cellular caspase inhibition

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[a] Abbreviations: ADP, adenosine diphosphate; DMF, dimethyl formamide; *m*-CPBA, *meta*-chloroperbenzoic acid; NBS, *N*-bromosuccinimide; PET, positron emission tomography; SD, standard deviation; SPECT, single photon emission computed tomography; $TBAH_2F_3$, tetrabutylammonium dihydrogen trifluoride; TsCl, toluenesulfonic acid chloride.

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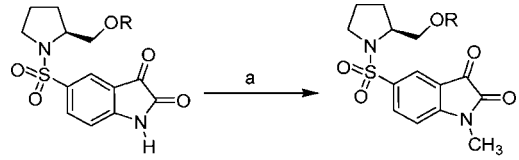
studies indicated that the 2-methoxymethyl compound (R = CH_3) seem to possess a higher biochemical caspase inhibition potency in intact apoptotic cells in comparison with the phenoxymethyl-modified compounds (R = Ph).[30] The *N*-1-position in lead structure **1** offers a potential site for structural modifications, thereby providing an excellent direction not only in generating a library of potential caspase inhibitors but also in targeting the potential precursors for radiolabeling. However, our idea was to concentrate on the synthesis of fluorinated analogues of **1** and to evaluate the influence of fluorine introduction on the caspase inhibition potency. Our motivation was based on the premise that introduction of a fluorine substituent may further enhance the potency of the derivative as compared to the lead **1**[28] and in turn would facilitate the identification of a target compound for radiofluorination. Accordingly, continuing our earlier work,[36] different fluorinated derivatives of **1** were synthesized with modifications at the *N*-1-position and their caspase inhibition potencies for caspase-1, -3, -6, and -7 were evaluated in vitro.

Scheme 1[a]



[a] (a) MeI, K_2CO_3, DMF.[28]

A number of these compounds after radiolabeling might also be suitable for monitoring of apoptosis by PET. Here, our radiofluorination methodology was focused on methods that were suitable for the fast introduction of the [^{18}F]fluorine radionuclide yielding isatin variants based on fluorohydrins. Fluorine-18 has a half-life of 110 min and a low positron energy, resulting in a higher spatial resolution of images compared to other light isotopes such as ^{11}C ($t_{1/2}$ = 20 min), ^{13}N ($t_{1/2}$ = 10 min), and ^{15}O ($t_{1/2}$ = 2 min) used in PET.[37] Thus, as a proof-of-concept, we succeded in the synthesis of a first-in-this-series fluorine-18 labeled model compound ([^{18}F]**37**).

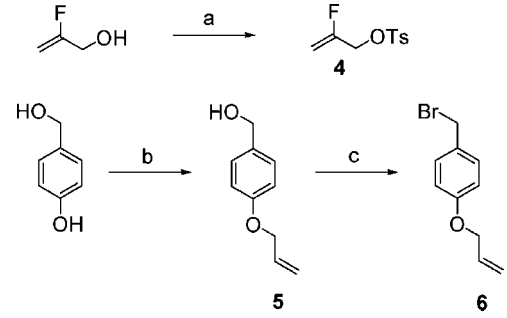
Results and Discussion

Chemistry. Our chemistry basically concentrated on employing fluorination techniques like bromofluorination (of precursor olefins) and epoxide ring-opening reactions toward vicinal fluorohydrins, which are rarely used for radiofluorination at present but are compatible with the structural elements of pyrrolidinylsulfonyl isatins (see discussion in the Radiochemistry section). The nonfluorinated compounds **1**, **2**, and **3** were recently identified as potent compounds with low IC_{50} values for caspases-3 and -7 and were therefore, earmarked as the standard compounds for comparing the results obtained from our compounds.[28,33,38] Accordingly, it was decided to incorporate structural variants at the *N*-1 position, which was proven to tolerate structural modifications without loss of caspase inhibition potency.[28,31] Consequently, a number of substituents with varying carbon units were attached to *N*-1, i.e., different precursor olefins (compounds **7**, **9**, **12**, **15**, **18**, and **20**) and precursor terminal epoxides (compounds **28**, **30**, **33**, **36**, and **38**). The new fluorinated isatin analogues were then prepared from these precursors by bromofluorination[39] and epoxide ring-opening with different amine/HF reagents.[40] While the lead compound **1** was obtained in seven steps starting from isatin and L-proline as described in a previous work,[28] its structurally related analogues, compounds **7**−**22** and **28**−**41**, were synthesized by alkylation of **1** and subsequent fluorination with the above-mentioned methods of organofluorine chemistry.

Bromofluorinations. Halofluorination of unsaturated compounds is a versatile method to introduce fluorine and, in addition, a second reactive function into organic compounds.[41−46] One of the most common procedures for bromofluorination uses *N*-bromosuccinimide (NBS) as the source of the bromonium ion and different amine−HF complexes.[46,47] A detailed account of regioselectivity of bromofluorination of functionalized olefins was provided by our research group a few years ago.[48] Furthermore, Katzenellenbogen et al. have previously demonstrated that bromofluorination could also serve as a radiochemical tool for introducing fluorine-18 into medicinally relevant compounds and thereby facilitate their use as imaging agents for PET.[49−51]

We started investigating the regio- and stereochemistry of bromofluorination of different terminal olefins derived from lead compound **1**. To our knowledge, this is the first instance of such methodology being applied to derivatize isatin compounds. In consideration of aforementioned aspects, we synthesized different alkenyl derivatives of **1** (**7**, **9**, **12**, **15**, **18**, and **20**) using a variety of alkenyl halides as electrophiles. Some of the alkenylation reagents were not commercially available and were prepared according to Scheme 2. 2-Fluoroallyl tosylate (**4**)[52] was synthesized in 90% yield from 2-fluoroallyl alcohol[53] and tosyl chloride using NaOH/Et_2O. *p*-Allyloxybenzyl bromide (**6**) was prepared in two steps starting from the commercially available *p*-hydroxybenzyl alcohol. However, no attempts were made to isolate intermediate **6** in pure and the crude product obtained after flash chromatography was directly used for the next step.

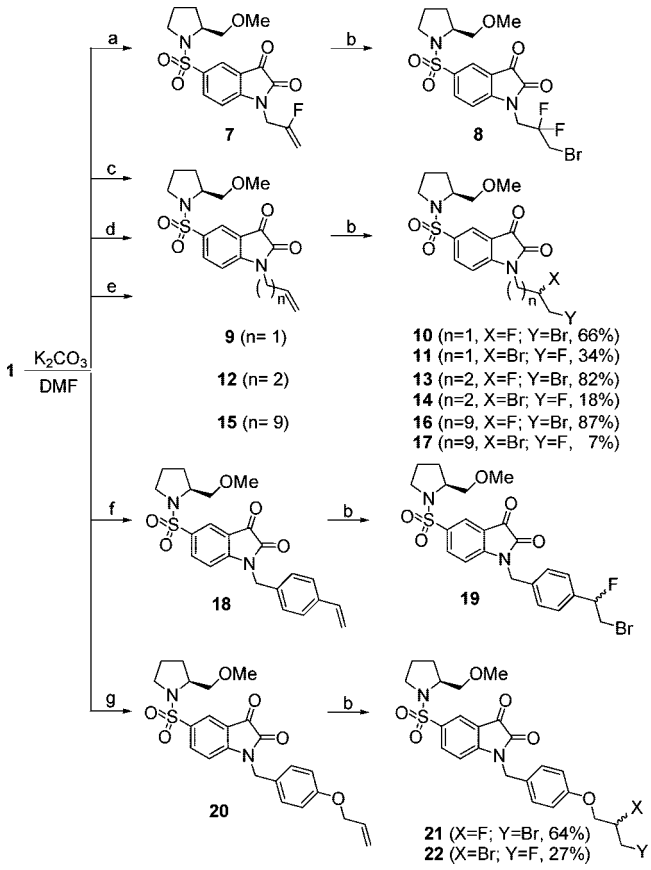
Scheme 2[a]



[a] (a) TsCl, NaOH, Et_2O; (b) KOH, Allyl bromide, DMF; (c) CBr_4, Ph_3P, dry CH_2Cl_2.

Bromofluorination of the terminal olefins (Scheme 3) afforded the bromofluorides in 52−87% isolated yields, and their regioisomeric and diastereomeric ratios were deduced from ^{19}F NMR studies of the crude reaction mixtures. All the experiments furnished some interesting results with respect to the regiochemistry of the resulting bromofluorides. Regardless of the strong electron withdrawing (−I) effect exerted by the isatin-sulfonamide core on the *N*-alkenyl moiety, which in allylic position disfavored a potential secondary cationic center; the regioselectivity of the bromofluorides though varied with increasing carbon chain length was always in favor of the secondary fluoride over the primary counterparts. While bromofluorination of **9** yielded a secondary bromofluoride favoring regioisomeric ratio of 2:1, the result was 4.5:1 in the case of bromofluorination of compound **12**. Similarly, compound **15** also yielded a secondary bromofluoride favoring regioisomeric ratio of 12:1.

Scheme 3[a]



[a] (a) compound **4**; (b) crystallized *N*-bromosuccinimide, Olah's reagent, dry CH_2Cl_2; (c) allyl bromide; (d) 4-bromobut-1-ene; (e) 11-bromoundec-1-ene; (f) 4-vinylbenzyl chloride; (g) compound **6**.

In the case of olefins **7** and **18**, the corresponding bromofluorides **8** and **19** were obtained with 100% regioselectivity. In case of compound **7**, this could be explained by the strong +M effect of the vinylic fluoride that stabilized the positive charge in the α-position and hence led to the exclusive formation of the geminal difluoride. On the other hand, compound **19** owes its exclusive formation to the stabilization of positive charge at the benzylic carbon. In the case of bromofluorination of **20**, the allylic oxygen destabilized the secondary cationic position and hence a significant amount of **21** was formed in addition to **22**.

Practically no diastereoselectivity (with regard to the stereocenter in the pyrrolidine core) was witnessed in either of the regioisomers of any of the bromofluorinated products, with the exception of compound **16**, which possessed a diastereomeric ratio of 83:17 as observed by ^{19}F NMR. It might be that the long chain folds below the plane of isatin core, blocking one face of the double bond, leading to such diastereoselection.

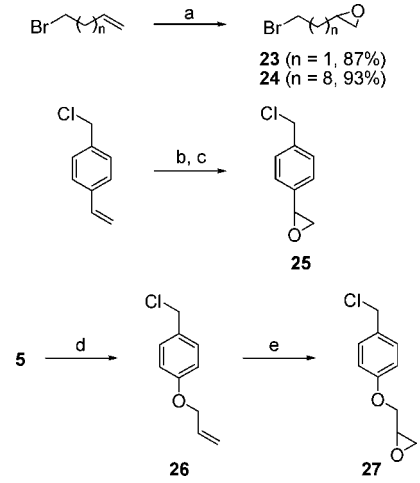
Considering the inhibitory activity (see section on enzyme assays) of the synthesized bromofluorides, the above results indicated that bromofluorination could be adopted as a powerful tool for future radiofluorinations and efforts in this direction are underway in our research group.

Synthesis of Fluorohydrins from Epoxides. In recent years, fluorohydrins[40,54] have attracted much attention in organofluorine chemistry[55] as they act as precursors in the synthesis of biochemically active, monofluorinated analogues of natural products[56,57] such as steroids,[58] amino acids,[59] and carbohydrates.[60−62] One of the main reasons for unique biological properties of vicinal fluorohydrins could be attributed to the fact that the presence of fluorine at vicinal position to the free hydroxyl group increases the acidity of hydroxyl function and hence influences its ability to act as hydrogen bond donor and/or acceptor. So, when biochemically active fluorohydrins are administered into the body, they may show enhanced activity against their in vivo targets (enzymes/receptors) compared to their nonfluorinated counterparts due to modified cell permeability and biodistribution.[63−65]

The most extensively used method for the synthesis of vicinal fluorohydrins is the *anti*-selective ring-opening of epoxides with different fluorinating agents,[66,67] which generally have different selectivities with the same oxirane. Ring-opening can occur by different reaction mechanisms, S_N1 or S_N2, explaining the selectivity of the formal addition of HF. Carbenium ion-stabilizing substituents favor an S_N1-type reaction, whereas destabilizing substituents favor an S_N2 pathway.[41,68] In recent years, we systematically investigated the selectivity of different hydrofluorinating reagents in the ring-opening of terminal and bicyclic epoxides including first asymmetric ring-opening by hydrofluorinating agents.[69,70] As fluoride donors, Olah's reagent, $Et_3N\cdot 3HF$, and KHF_2 were mostly used.[71,72]

On the basis of the above background information, we started investigating the regio- and stereochemistry of ring-opening of different terminal epoxides (enantiopure and racemic) derived from lead compound **1**. The objective (as with bromofluorides) was to synthesize nonradioactive fluorohydrins to evaluate their caspase inhibition potency and then to select candidate compounds with excellent inhibition potencies for the downstream caspases-3 and -7, for the radiochemical resynthesis via ^{18}F-labeling. Accordingly, we synthesized different terminal epoxides **28**, **30**, **33**, **36**, and **38** using a variety of epoxyalkyl halides as electrophiles. Most of the alkylation reagents were not commercially available and were prepared according to Scheme 4.

Scheme 4[a]

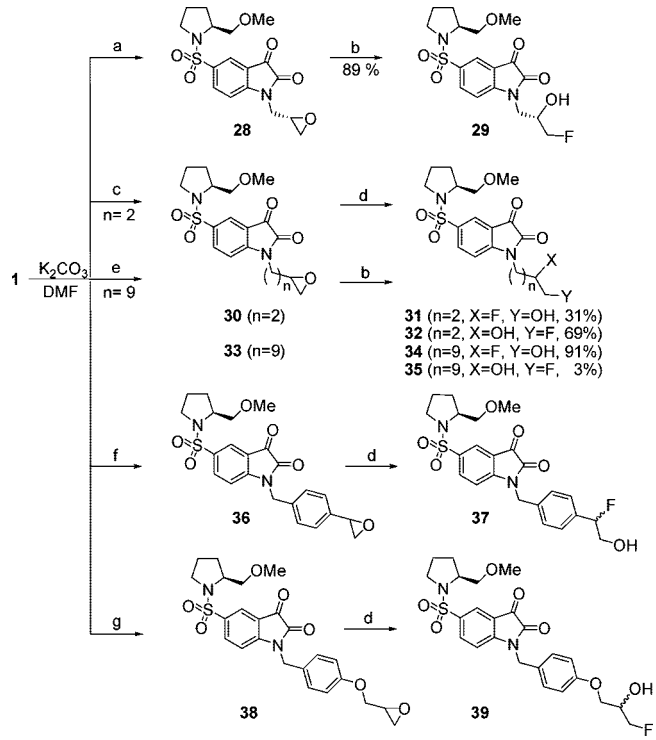


[a] (a) *m*-CPBA, CH_2Cl_2; (b) NBS, acetone−water (7:3); (c) powdered NaOH; (d) $SOCl_2$, CH_2Cl_2; (e) *m*-CPBA, CH_2Cl_2.

In our investigation of regioselective ring-opening of epoxides with hydrofluorinating agents, another objective was the synthesis of fluorohydrins within a short time in order to transfer the investigated methology to ^{18}F-radiochemistry. Keeping this in view, most of the synthesized epoxides were initially opened with Olah's reagent, which predominantly yielded the regioisomer formed from the more stable carbocation (S_N1-like reaction), although there is no evidence for the formation of a free cationic center.[73,74] The regioisomeric and diastereomeric

ratios of resulting fluorohydrins were then deduced from ^{19}F NMR studies of the crude reaction mixtures (Scheme 5).

Scheme 5[a]

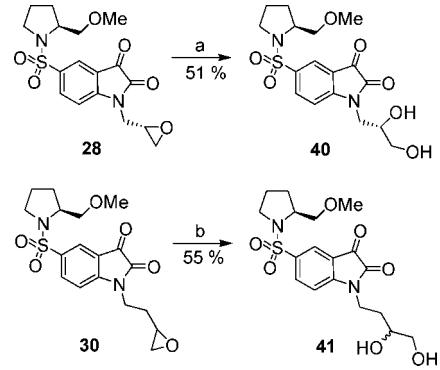


[a] (a) (*S*)-(+)-glycidyl nosylate; (b) Olah's reagent, CH_2Cl_2; (c) **23**; (d) $Et_3N \cdot 3HF$; (e) **24**; (f) **25**; (g) **27**.

All the experiments provided some interesting results with respect to the regioselectivity of the resulting fluorohydrins. Initially, compound **28** was chosen as the substrate to explore the above-mentioned chemistry. When **28** was treated with Olah's reagent, the results were totally surprising in the sense that the reaction yielded the regioisomer with fluorine at the primary position (instead of the secondary position) with 99% selectivity and 51% isolated yield. This could be attributed to the strong electron withdrawing (−I) effect exerted by the isatinsulfonamide core on the *N*-alkyl moiety that destabilizes a potential secondary carbocationic center. Owing to the strong acidity of Olah's reagent, the reaction also yielded about 10% of oligomers as the side products. Because the explored reaction time (12−14 h) was too long, ring-opening was also tried out in neat $Et_3N \cdot 3HF$ at 80−90 °C, but the reaction time in this case was even longer (21−22 h). To reduce the reaction time, many experiments were carried out with the main focus on (i) optimization of Olah's reagent amounts, (ii) varying pyridine to HF ratios by definite addition of pyridine with an aim to increase the nucleophilicity of fluoride, and (iii) reaction temperature (some reactions were also carried out at 45 °C). However, none of the above experiments could reduce the reaction time below 8 h. These results pointed out that C_3 epoxide **28** may be unsuitable for radiofluorination. Therefore, variations of the carbon chain length (of epoxide) on the reaction time and regioselectivity were explored. Accordingly, reactions were carried out with racemic C_4 epoxide **30** and racemic long chain (C_{11}) epoxide **33**. While **33** yielded the expected secondary fluorohydrin favoring regioisomeric ratio of 30:1, fluorohydrin formation from **30** and Olah's reagent under different reaction conditions was not observed. Therefore, ring-opening of **30** was achieved with neat $Et_3N \cdot 3HF$, which provided the fluorohydrins **31** and **32** in 71% yield. Here, the formation of the primary fluoride **32** is favored (regioisomeric ratio 2.2:1). Similarly, the racemic aromatic epoxides **36** and **38** were also opened with neat $Et_3N \cdot 3HF$. The styrene oxide derivative **36** yielded the secondary fluorohydrin **37** not only with 100% regioselectivity but also in considerably reduced reaction time (1.5−2 h). This result could be attributed to the activated benzylic position in **36** for attack by the nucleophilic fluoride. Although up to 38% of oligomers were also detected (^{19}F NMR), the isolated yield of 51% was still reasonable. Likewise, the reaction of the phenylglycidether **38** with $Et_3N \cdot 3HF$ yielded the primary fluorohydrin **39** with more than 99% regioselectivity after 14−16 h of reaction time. Because the strong electron withdrawing (−I) effect of the phenoxy moiety destabilized the secondary carbocationic center, the incoming fluoride ion was forced to attack at the terminal carbon in S_N2 pathway, leading to the predominance of primary fluoride **39** and increased reaction time (14−16 h). Compound **39** was obtained in 45% yield, but 33% of oligomers were also detected in the crude product by ^{19}F NMR spectroscopy. No diastereoselectivity was observed in either of the regioisomers of any of the fluorohydrins except **29**, which is diastereopure, and **34**, which had a diastereomeric excess of 82% (^{19}F NMR).

To compare the caspase inhibition potency of synthesized fluorohydrins with the corresponding diols, two exemplary diols were prepared (Scheme 6).

Scheme 6[a]



[a] (a) Olah's reagent, CH_2Cl_2, saturated $NaHCO_3$ soln; (b) silica gel.

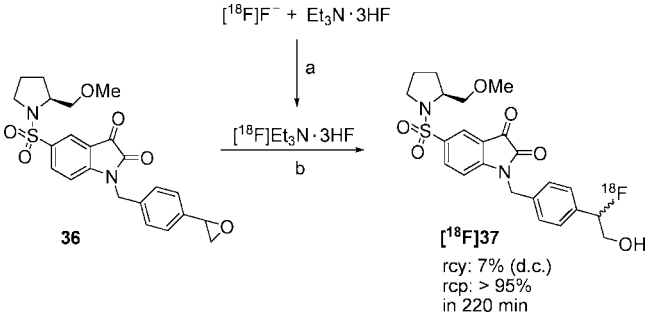
Accordingly, synthesis of enantiopure diol **40** from **28** was realized with Olah's reagent and saturated sodium bicarbonate solution in 51% yield. Also, racemic diol **41** was synthesized in 55% yield by ring-opening of the precursor epoxide **30** on a silica gel column followed by elution of the product with 5% $MeOH/CH_2Cl_2$.

Comprehensive scrutiny and analysis of the obtained results in combination with the data obtained by the enzyme assays prompted us to devise a potential radiofluorination of the racemic styrene oxide derivative **36**. Short reaction time, remarkable regioselectivity, and excellent caspase-3 and -7 inhibition potency (see below) were the reasons for selecting compound **36** as the precursor compound for ^{18}F-fluorination.

Radiochemistry. The synthesis of [^{18}F]**37** was evaluated with different fluorination reagents. Initial experiments with Kryptofix 2.2.2 (K 222) and [^{18}F]KF resulting in [^{18}F]K(K 222)F yielded only traces of fluorohydrin [^{18}F]**37** under basic reaction conditions (K_2CO_3). Very recently, Zhou et al.[75] observed that the effectiveness of the labeling procedure with the system K_2CO_3/K 222/[^{18}F]fluoride ion/isatin derivative depends among other parameters on the amount of base (K_2CO_3) in the reaction. Under their labeling conditions, not less than 2 mg of K_2CO_3 is necessary to obtain a high yield of the labeled, ring-opened isatinate that was converted to the corresponding isatin under acidic conditions. Our labeling protocol uses much more K_2CO_3, indicating that the low radio-

chemical yield of [^{18}F]**37** is caused by other factors. Obviously, basic reaction conditions are not suitable to open the epoxide ring of **36**. However, the utilization of [^{18}F]poly(hydrogen fluoride) pyridinium ([^{18}F](HF)$_n$·pyridine) prepared as previously described[76] provided a radiochemical yield of up to 5% (decay corrected, d.c.) for [^{18}F]**37**. Furthermore, the radiochemical yield of [^{18}F]**37** was increased and optimized to 7% (d.c.) when [^{18}F]triethylamine trihydrofluoride ([^{18}F]Et$_3$N·3HF) was used for radiofluorination (Scheme 7). For the first time, a nucleophilic fluorinating reagent generated from Et$_3$N·3HF and [^{18}F]F$^-$ via isotopic exchange was successfully applied in the synthesis of the target ^{18}F-labeled compound [^{18}F]**37**.

Scheme 7[a]



[a] (a) CH_3CN, Δ, sonication; (b) CH_3CN, Δ.

In the investigations by Zhou et al.,[75] the application of "no-carrier-added" concept prevented the formation of the desired labeled isatin substitution product in certain cases. This was due to the consumption of [^{18}F]fluoride caused by the nucleophilic attack and reversible addition of this species at the C-3 carbonyl group of the precursor that was used in a huge excess compared to [^{18}F]fluoride. In contrast to this, our applied carrier-added method reverting to an excess of [$^{18/19}$F]fluoride (in the form of [$^{18/19}$F]Et$_3$N·3HF) yielded the desired labeled nucleophilic substitution product [^{18}F]**37**. Apparently, the completely different stoichiometric ratios of fluoride to precursor amounts in our experiments as compared to the investigations by Zhou et al. account for the product formation in our labeling procedure and prevent it in the cited paper.[75] Compound [^{18}F]**37** was finally obtained in 220 min with a radiochemical purity of >95%, showing moderate specific activities (<1 GBq/μmol) that correspond with the here chosen carrier-added radiofluorination approach. Such low specific activities may limit the application of [^{18}F]**37** for in vivo imaging of activated caspases and therefore, future investigations will aim at increasing the specific activity (e.g., by the reduction of carrier addition and increase of starting activity).

Although the radiochemical yield is low, our new fluorine-18 source ([^{18}F]Et$_3$N·3HF) allows us to carry out the radiosynthesis of [^{18}F]**37** by utilizing [^{18}F]KF. This success could be attributed to the fact that [^{18}F]Et$_3$N·3HF is an acidic fluorinating agent where the basicity and nucleophilicity of [^{18}F]F$^-$ is considerably reduced when compared to [^{18}F]KF, which in turn is due to the intermolecular hydrogen bonding. Contrary to the investigations by Zhou et al.,[75] under such conditions of reduced nucleophilicity and increased acidity of the fluorination reagent, the nucleophilic addition of [^{18}F]F$^-$at the C-3 carbonyl group of **36** and isatinate intermediate formation by the ring-opening of isatin are less likely to occur. The above result clearly highlights the improved efficacy of the less basic [^{18}F]Et$_3$N·3HF for radiolabeling experiments when compared to [^{18}F]KF, as far as the S_N reactions with [^{18}F]F$^-$ in isatin compounds are concerned.

Enzyme Assays. The caspase inhibition potencies of all the synthesized analogues of 5-pyrrolidinylsulfonyl isatin (comprising of potential radiolabeling precursors **7**, **9**, **12**, **15**, **18**, **20**, **28**, **30**, **33**, **36**, and **38**; the nonradioactive counterparts of potential caspase-targeted radioligands **8**, **10**+**11**, **13**+**14**, **16**+**17**, **19**, **21**+**22**, **29**, **31**+**32**, **34**+**35**, **37**, and **39**, together with some important nonfluorinated derivatives **40** and **41**, were tested for their inhition potencies against caspases-1, -3, -6, and -7 by using fluorogenic in vitro caspase inhibition assays. The resulting caspase inhibitory values were obtained as IC_{50} values by a nonlinear regression fit of the concentration-dependent reaction rates and the in vitro results have been captured in Table 1. According to Lee et al.[28,33] compounds **1**, **2**, and **3a** are potent caspase inhibitors. To directly compare our findings, the inhibition potencies of compounds **1**, **2**, and **3a** have also been tabulated. Most of the target compounds exhibited nanomolar binding potency for inhibiting the effector caspases, caspase-3 and caspase-7. Normally, no inhibition of caspase-1 and caspase-6 was observed for any of the compounds.

Table 1. Caspase Inhibition Potencies of the 5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin Analogues for Caspases-1, -3, -6, and -7 Expressed as IC_{50}Values

	IC_{50}, nM[a]			
compd	caspase-1	caspase-3	caspase-6	caspase-7
1	>500000	84.9 ± 25.6	>500000	1290 ± 466
2	>500000	16.9 ± 7.7	>500000	294 ± 178
3a	>500000	2.4 ± 0.3	>500000	304 ± 73
7[b,c]	nd	499 ± 239	nd	266 ± 96
8[b]	nd	1880 ± 1440	nd	1660 ± 310
9	>500000	25 ± 16	>500000	24 ± 11
10+**11**[b] (~2:1)	>500000	26 ± 19	>500000	15 ± 8
12	>500000	31 ± 13	>500000	50 ± 16
13+**14**[b] (82:18)	>500000	96 ± 32	>500000	20 ± 7
15	>500000	74 ± 20	>500000	111 ± 26
16+**17**[b] (~11:1)	>500000	34 ± 9	>500000	86 ± 6
18	>500000	9.3 ± 4.3	>500000	7.2 ± 2.4
19[b]	>500000	43 ± 19	>500000	16 ± 2
20	>500000	10 ± 0.5	>500000	0.6 ± 0.2
21+**22**[b] (~7:3)	>500000	26 ± 2	>500000	1.9 ± 0.2
28	3160 ± 490	17 ± 5	>500000	9.0 ± 1.3
29[b]	>500000	19 ± 12	>500000	349 ± 33
30	>500000	151 ± 62	>500000	68 ± 8
31+**32**[b] (1:1)	>500000	12 ± 6	>500000	214 ± 5
32[b]	>500000	3.6 ± 1.0	>500000	99 ± 32
33	>500000	23 ± 11	>500000	61 ± 13
34+**35**[b] (91:9)	>500000	47 ± 6	>500000	63 ± 17
36	>500000	6.6 ± 1.4	>500000	1.9 ± 0.6
37[b]	>500000	80 ± 8	>500000	7.6 ± 0.1
38	>500000	42 ± 15	>500000	2.4 ± 0.2
39[b]	>500000	145 ± 5	>500000	5.0 ± 0.6
40	>500000	11 ± 4	>500000	43 ± 4
41	>500000	275 ± 40	>500000	61 ± 12

[a] Values are the mean ± SD of three assays. [b] Nonradioactive counterparts of PET-compatible caspase-targeted radioligands. [c] Taken from ref 36.

Recently, we and Chu et al. have also modified the *N*-1 position of the isatins by synthesizing a variety of *N*-benzyl derivatives and got encouraging results.[30,31] The above results clearly imply that the *N*-1 position of the 5-pyrrolidinylsulfonylisatins is a potential site for modification and development of isatins as caspase-targeted radioligands.

Discussion

The IC_{50}-values for caspase inhibition of all compounds revealed some striking observations. It was reported earlier that isatin sulfonamides are highly potent and selective nonpeptide based inhibitors of the effector caspases-3 and -7. Our results have further reinforced the fact that 5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatins are potent inhibitors, which could be used as radiotracers for molecular imaging of activated caspases in apoptosis. All compounds except the *gem*-difluoride **8** exhibited moderate to high inhibition potencies for caspases-3

and -7. All compounds also exhibited very low potencies for caspases-1 and -6, with the exception of compound **28**, which was a weak inhibitor of caspase-1. Among the various terminal olefins **7**, **9**, **12**, **15**, **18**, and **20**, which were also the precursors for the corresponding bromofluorides, compound **18** was the most active against caspase-3 (IC_{50}: 9.3 nM) and compound **20** was the most active against caspase-7, with a subnanomolar IC_{50} value (0.6 nM). Similarly, having a closer look at the derived bromofluorides **8**, **10**+**11**, **13**+**14**, **16**+**17**, **19**, and **21**+**22**, we observed that among this series a mixture of regioisomeric bromofluorides **21** and **22** (∼7:3) was the most active against both caspase-3 (IC_{50}: 26 nM) and caspase-7 (IC_{50}: 1.9 nM). A mixture (92:8) of compounds **16** and **17** also showed good activity against these caspases. Because all the bromofluorides possessed very good activity against caspase-3 and -7, they provide a new class of isatin derivatives with excellent potential for radiolabeling and might be used as caspase-targeted radioligands for apoptosis imaging. Further efforts, i.e., to synthesize pure isomers, are underway.

On analyzing the third series of compounds, namely the precursor terminal epoxides **28**, **30**, **33**, **36**, and **38**, we observed that among this series, the styrene oxide derivative **36** was the most active against both caspase-3 (IC_{50}: 6.6 nM) as well as caspase-7 (IC_{50}: 1.9 nM). Similarly, comprehensive analysis of the corresponding fluorohydrins **29**, **31**+**32**, **34**+**35**, **37**, and **39** revealed that regioisomer **32** (primary fluoride) of C_4 fluorohydrins was the most active against caspase-3, and compound **39** was the best inhibitor for caspase-7 within this particular series. Generally, the *N*-benzyl compounds showed the lowest IC_{50} values. Also, the binding potencies of the bromofluorides and fluorohydrins were not significantly influenced by their regiochemistry. Because all the fluorohydrins exhibited very good activity against caspases-3 and -7, they (like bromofluorides) offer a new class of isatin derivatives with tremendous potential for radiofluorination. However, it should be noted that the short-chain fluorohydrins **29** (17:1) and **32** (28:1) are better inhibitors for caspase-3, while the benzyl compounds **37** (1:11) and **39** (1:29) are much more active as inhibitors of caspase-7. The long-chain fluorohydrins **34**/**35** are almost equal in their activity for both the caspases (Table 1). Accordingly, as a proof-of-concept, the ^{18}F-labeled isatin [^{18}F]**37** was synthesized as a first fluorohydrin model compound whose nonradioactive counterpart **37** possessed high inhibition potency, comparable to the activity of compounds prepared earlier.[30,32]

As far as the miscellaneous compounds were concerned, the C_3 enantiopure diol **40** was more active than its C_4 racemic counterpart **41**. Interestingly, **40** was also more active than its fluorohydrin counterpart **29**.

Conclusion

We were successful in synthesizing two new series of isatin derivatives (the bromofluorides and fluorohydrins), and their inhibition potencies were evaluated in vitro by biochemical caspase inhibition assays. In line with previous findings, the majority of synthesized 5-pyrrolidinylsulfonyl isatins exhibited high potency for inhibiting the downstream effector caspases-3 and -7. The obtained results have added a new dimension to the synthesis and development of nonpeptide-based inhibitors of caspase-3 and -7 and also enabled us in expanding the range of the existing class of such inhibitors. A potential caspase-targeted radioligand should selectively detect one of the activated caspases in vivo. For these reasons, as a proof-of-concept, the radiotracer [^{18}F]**37** was synthesized as a first fluorohydrin-based PET-compatible caspase-targeted model tracer. Aware of the low specific activity of [^{18}F]**37**, other precursors are also being explored for immediate radiolabeling in order to establish potent compounds that could well serve as "chemical solutions" to the biological problem of imaging apoptosis.

Experimental Section

General Methods. Chemistry and Radiochemistry. All the chemicals, reagents, and solvents for the synthesis of compounds were analytical grade and used without further purification, unless otherwise specified. ^{1}H NMR (300.13 MHz, 400.13 MHz, 500 MHz and, 600 MHz), ^{13}C NMR (75.5 MHz, 100.63 MHz, 125.5 MHz, and 150.66 MHz,) and ^{19}F NMR (282.4 MHz) spectra were recorded in $CDCl_3$ with TMS for ^{1}H NMR, $CDCl_3$ for ^{13}C NMR, and $CFCl_3$ for ^{19}F NMR as the internal standards. All chemical shift values were recorded in ppm (δ). Exact mass analyses were conducted on a Waters Quattro LC and a Bruker MicroTof apparatus. All these spectroscopic and analytical investigations were done by staff members of the Organic Chemistry Institute, University of Münster. Silica coated aluminum foils (silica gel 60 F_{254}) from MERCK with 0.2 mm layer thickness were used for thin layer chromatography (TLC). Column chromatography was generally performed on silica gel (60−120 mesh) using suitable solvent mixtures. The purity of compounds was proved by HPLC comprised of a Knauer system with two K-1800 pumps, an S-2500 UV detector, and a Eurospher 100 C_{18} reverse phase column (250 mm × 4.6 mm). Detection was conducted at λ = 254 nM. The solvents used were: A (water + 0.1% TFA) and B (CH_3CN + 0.1% TFA). The mobile phase was a gradient using 90:10 A/B to 20:80 A/B mixture over 30 min, holding for 5 min and back to 90:10 A/B mixture in 5 min at a common flow rate of 1.5 mL/min. Separation and purification of the radiosynthesized compound [^{18}F]**37** were performed by gradient radio-HPLC (radio-HPLC A) using a Knauer K-500 and a Latek P-402 pump, a Knauer K-2000 UV detector (λ = 254 nm), a Crismatec NaI(Tl) Scintibloc 51 SP51 γ-detector, and a Nucleosil 100−10 C_{18} column (250 × 8 mm^2). Sample injection was carried out using a Rheodyne injector block (type 7125 incl. 1000 μL loop). The recorded data were processed by the NINA version 4.9 software (GE Medical Systems—Functional Imaging GmbH).

The starting compound **1** was prepared according to a given literature protocol,[28] and 2-fluoroallyl tosylate **4** was prepared from 2-fluoroallyl alcohol according to literature.[52]

General Procedure for the Synthesis of *N*-Alkenyl Isatins. To a solution of (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) in dry DMF (3 mL), anhydrous potassium carbonate (2.5 equiv) was added under argon atmosphere and the reaction mixture was stirred at ambient temperature for 30 min. An excess of the alkylating agent, (2−3 equiv) was slowly added and the reaction mixture was stirred at ambient temperature for 12−14 h in the case of 2-fluoroallyl tosylate and simple alkyl halides but only for 6−8 h in the case of benzylic halides. Removal of the solvents in vacuo furnished the crude products, which were purified by silica gel chromatography.

(*S*)-1-(2-Fluoroallyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (7). The synthesis of this compound was already described in our earlier work.[36] HPLC t_R = 24.47 min (99.8%).

(*S*)-1-Allyl-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (9). (*S*)-5-[1-(2-Methoxymethyl-pyrrolidinyl)sulfonyl]isatin (**1**) (400 mg, 1.23 mmol) was converted to **9** using anhydrous K_2CO_3 (425 mg, 3.08 mmol) and allyl bromide (0.22 mL, 2.46 mmol), as described in the general procedure, and stirred for 14 h. The crude product was purified by column chromatography (ethyl acetate:cyclohexane, 9:1) to yield a deep-orange colored gummy solid. Yield: 320 mg (71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50−1.68 (m, 2H), 1.80−1.98 (m, 2H), 3.04−3.17 (m, 1H), 3.33−3.56 (m, 2H), 3.35 (s, 3H), 3.58 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.68−3.79 (m, 1H), 4.43 (dd, 2H, $^3J_{H,Ha}$ = 5.5 Hz, $^4J_{H,Hc}$ = 0.6 Hz), 5.36 (m, 1H), 5.37 (m, 1H), 5.85 (ddt, 1H, $^3J_{Ha,H}$ = 5.5 Hz, $^3J_{Ha,Hb}$ = 17.3 Hz, $^3J_{Ha,Hc}$ = 10.3 Hz), 7.05 (d, 1H, $^3J_{H,H}$ = 8.4 Hz), 8.04 (d, 1H, $^4J_{H,H}$ = 1.9 Hz), 8.07 (dd, 1H,$^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 1.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.9, 42.9, 49.4, 59.1, 59.2, 74.9,

111.1, 117.4, 119.4, 124.5, 129.7, 136.2, 137.4, 153.4, 157.8, 181.8. HRMS (ESI-MicroTof): *m/e* 419.1242 (M + Na + CH_3OH)$^+$, calcd for $C_{18}H_{24}N_2NaO_6S$ 419.1247. HPLC t_R = 24.28 min (99.6%).

(*S*)-1-(But-3-enyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (12). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-isatin (**1**) (500 mg, 1.54 mmol) was converted to **12** using anhydrous K_2CO_3 (532 mg, 3.85 mmol) and 4-bromo-1-butene (0.3 mL, 3.08 mmol). as described in the general procedure, and stirred for 14 h. The crude product was purified by column chromatography (CH_2Cl_2:MeOH, 98:2) to yield a deep-orange colored gummy solid. Yield: 531 mg (91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.64−1.74 (m, 2H), 1.85−1.96 (m, 2H), 2.52 (q, 2H, $^3J_{H,H}$ = 7.0 Hz), 3.10−3.18 (m, 1H), 3.34−3.47 (m, 2H), 3.36 (s, 3H), 3.60 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.71−3.77 (m, 1H), 3.88 (t, 2H, $^3J_{H,H}$ = 7.0 Hz), 5.08−5.19 (m, 2H), 5.85 (ddt, 1H, $^3J_{Ha,H}$ = 6.9 Hz, $^3J_{Ha,Hb}$ = 9.8 Hz, $^3J_{Ha,Hc}$ = 16.8 Hz), 7.06 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.04 (d, 1H, $^4J_{H,H}$ = 1.7 Hz), 8.10 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 31.6, 40.0, 49.4, 59.1, 59.2, 74.8, 110.5, 117.3, 118.6, 124.5, 133.4, 133.7, 137.4, 153.6, 157.8, 182.0. HRMS (ESI-MicroTof): *m/e* 433.1411 (M + Na + CH_3OH)$^+$, calcd for $C_{19}H_{26}N_2NaO_6S$ 433.1415. HPLC t_R = 26.48 min (98.9%).

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-(undec-10-enyl)isatin (15). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-isatin (**1**) (500 mg, 1.54 mmol) was converted to **15** using anhydrous K_2CO_3 (532 mg, 3.85 mmol) and 11-bromo-1-undecene (0.67 mL, 3.08 mmol), as described in the general procedure, and stirred for 14 h. The crude product was purified by column chromatography (CH_2Cl_2:MeOH, 97:3) to yield a light yellow colored gummy solid. Yield: 660 mg (90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.28−1.73 (m, 16H), 1.90−1.94 (m, 2H), 2.05 (q, 2H, $^3J_{H,H}$ = 6.8 Hz), 3.11−3.17 (m, 1H), 3.36 (s, 3H), 3.37−3.47 (m, 2H), 3.60 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.74−3.78 (m, 3H), 4.94 (dd, 1H, $^2J_{Hb,Hc}$ = 1.0 Hz, $^3J_{Hb,Ha}$ = 10.2 Hz), 5.01 (dd, 1H, $^2J_{Hc,Hb}$ = 1.4 Hz, $^3J_{Hc,Ha}$ = 17.1 Hz), 5.85 (ddt, 1H, $^3J_{Ha,H}$ = 6.7 Hz, $^3J_{Ha,Hb}$ = 10.2 Hz, $^3J_{Ha,Hc}$ = 16.9 Hz), 7.02 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.04 (d, 1H, $^4J_{H,H}$ = 1.5 Hz), 8.10 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 26.9, 27.2, 28.8, 28.9, 29.0, 29.2, 29.3, 29.4, 33.8, 40.7, 49.4, 59.1, 59.2, 74.8, 110.4, 114.2, 117.4, 124.5, 133.6, 137.5, 139.1, 153.7, 157.8, 182.2. HRMS (ESI-MicroTof) *m/e*: 531.2482 (M + Na + CH_3OH)$^+$, calcd for $C_{26}H_{40}NaN_2O_6S$ 531.2489. HPLC t_R = 38.73 min (98.9%).

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-(4-vinylbenzyl)isatin (18). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-isatin (**1**) (100 mg, 0.3 mmol) was converted to **18** using anhydrous K_2CO_3 (106 mg, 0.77 mmol) and 4-vinylbenzyl chloride (0.09 mL, 0.617 mmol), as described in the general procedure, except that 4-vinylbenzyl chloride was added at 0 °C and the reaction mixture stirred at the same temperature for 30 min and then at ambient temperature for 7 h. The crude product was purified by column chromatography (ethyl acetate:toluene 2:3) to yield a deep-orange colored gummy solid. Yield: 112 mg (83%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60−1.72 (m, 2H), 1.79−1.96 (m, 2H), 3.04−3.15 (m, 1H), 3.32−3.44 (m, 2H), 3.33 (s, 3H), 3.57 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.68−3.75 (m, 1H), 4.96 (s, 2H), 5.28−5.30 (dd, 1H, $^2J_{Hb,Hc}$ = 0.5 Hz, $^3J_{Hb,Ha}$ = 10.9 Hz), 5.75−5.78 (dd, 1H, $^2J_{Hc,Hb}$ = 0.6 Hz, $^3J_{Hc,Ha}$ = 17.6 Hz), 6.71 (dd, 1H, $^3J_{Ha,Hb}$ = 10.9 Hz, $^3J_{Ha,Hc}$ = 17.6 Hz), 6.92 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 7.30 (d, 2H, $^3J_{H,H}$ = 8.2 Hz), 7.41 (d, 2H, $^3J_{H,H}$ = 8.2 Hz), 7.97 (dd, 1H, $^3J_{H,H}$ = 8.9 Hz, $^4J_{H,H}$ = 1.9 Hz), 8.04 (d, 1H, $^4J_{H,H}$ = 1.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 44.2, 49.3, 59.0, 59.1, 74.8, 111.3, 114.9, 117.5, 124.5, 127.0, 127.8, 133.1, 134.0, 135.9, 137.4, 137.9, 153.3, 157.8, 181.8. HRMS (ESI-MicroTof): *m/e* 495.1581 (M + Na + CH_3OH)$^+$, calcd for $C_{24}H_{28}N_2NaO_6S$ 495.1560. HPLC t_R = 30.57 min (99.6%).

(*S*)-1-[4-(Allyloxy)benzyl]-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (20). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) (100 mg, 0.3 mmol) was converted to **20** using anhydrous K_2CO_3 (106 mg, 0.77 mmol) and a crude sample of **6** (139 mg, 0.617 mmol), as described in the general procedure, except that **6** was added at 0 °C and the reaction mixture stirred at the same temperature for 30 min and then at ambient temperature for 7 h. The crude product was purified by column chromatography (ethyl acetate:toluene 4.5:5.5) to yield a deep-orange colored gummy solid. Yield: 139 mg (90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.64−1.66 (m, 2H), 1.84−1.91 (m, 2H), 3.07−3.11 (m, 1H), 3.32−3.43 (m, 2H), 3.33 (s, 3H), 3.57 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.70−3.72 (m, 1H), 4.53 (dt, 2H, $^3J_{H,Ha}$ = 5.2 Hz, $^4J_{H,Hb+Hc}$ = 1.3 Hz), 4.90 (s, 2H), 5.31 (ddd, 1H, $^2J_{Hc,Hb}$ = 2.8 Hz,$^3J_{Hc,Ha}$ = 10.5 Hz, $^4J_{Hc,H}$ = 1.3 Hz), 5.43 (ddd, 1H, $^2J_{Hb,Hc}$ = 3.1 Hz, $^3J_{Hb,Ha}$ = 17.3 Hz, $^4J_{Hb,H}$ = 1.5 Hz), 6.07 (ddd, 1H, $^3J_{Ha,H}$ = 5.3 Hz, $^3J_{Ha,Hb}$ = 17.2 Hz, $^4J_{Ha,Hc}$ = 10.5 Hz), 6.90 (d, 2H, $^3J_{H,H}$ = 8.5 Hz), 6.94 (d, 1H,$^3J_{H,H}$ = 8.3 Hz), 7.26 (d, 1H, $^3J_{H,H}$ = 8.5 Hz), 7.99 (dd, 1H, $^3J_{H,H}$ = 8.1 Hz, $^4J_{H,H}$ = 1.1 Hz), 8.04 (d, 1H, $^4J_{H,H}$ = 1.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 28.8, 44.0, 49.3, 59.1, 59.2, 68.9, 74.8, 111.2, 115.4, 117.5, 118.0, 124.5, 125.8, 129.1, 132.9, 134.0, 137.4, 153.4, 158.0, 158.7, 182.0. HRMS (ESI-MicroTof): *m/e* 525.1646 (M + Na + CH_3OH)$^+$, calcd for $C_{25}H_{30}N_2NaO_7S$ 525.1666. HPLC t_R = 30.67 min (99.2%).

General Procedure for the Synthesis of *N*-(Bromofluoroalkyl) Isatins. To a dried, argon flushed Teflon vessel with a rubber septum, Olah's reagent (1.1−1.2 equiv) in dry methylene chloride (2 mL) was cooled to 0 °C. Using a syringe, a solution of terminal olefin **7**, **9**, **12**, **15**, **18**, or **20** in 5 mL of dry methylene chloride was injected dropwise along with gentle stirring. After the addition, crystallized *N*-bromosuccimide (NBS, 1−1.15 equiv) was added portionwise (5 portions) and the reaction mixture was stirred at 0 °C for 30 min and then at ambient temperature for 3−16 h, depending on the substrate. The reaction mixture was then cooled to 0 °C, quenched with chilled saturated sodium bicarbonate solution (20 mL), and the aqueous layer extracted completely with excess CH_2Cl_2. The combined organic extracts were sequentially washed with 1 N HCl solution (15 mL), followed by 5% sodium bicarbonate solution (15 mL), dried over $MgSO_4$, and filtered. The solvent was then removed in vacuo to afford the crude product, which was purified by silica gel chromatography.

(*S*)-1-(3-Bromo-2,2-difluoropropyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (8). (*S*)-1-(2-Fluoroallyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**7**) (70 mg, 0.183 mmol) was converted to **8** using Olah's reagent (0.05 mL, 0.219 mmol) and crystallized NBS (37.5 mg, 0.21 mmol), as described in the general procedure, and stirred for 16 h. The crude product was purified by column chromatography (ethyl acetate:toluene 1:1) to yield a deep-orange colored gummy solid. Yield: 48 mg (55%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65−1.73 (m, 2H), 1.87−1.94 (m, 2H), 3.10−3.18 (m, 1H), 3.35−3.46 (m, 2H), 3.36 (s, 3H), 3.58 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.69 (t, 2H, $^3J_{H,F}$ = 13.3 Hz), 3.73−3.79 (m, 1H), 4.39 (t, 2H, $^3J_{H,F}$ = 13.5 Hz), 7.19 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.07 (d, 1H, $^4J_{H,H}$ = 1.5 Hz), 8.10 (dd, 1H, $^3J_{H,H}$ = 8.4 Hz, $^4J_{H,H}$ = 1.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 28.8, 29.3 (t, $^2J_{C,F}$ = 30.7 Hz), 43.8 (t, $^2J_{C,F}$ = 28.5 Hz), 49.3, 59.1, 59.2, 74.8, 111.6, 117.6, 119.9 (t, $^1J_{C,F}$ = 247.4 Hz), 124.6, 134.7, 137.5, 152.8, 158.0, 180.4. ^{19}F NMR (282 MHz, $CDCl_3$): δ −100.4 (quintet, 2F, $^3J_{H,F}$ = 13.3 Hz). HRMS (ESI- MicroTof): *m/e* 535.0314 (M + Na + CH_3OH)$^+$, calcd for $C_{18}H_{23}BrF_2N_2NaO_6S$ 535.0320. HPLC t_R = 27.28 min (99.5%).

(*S*)-1-(3-Bromo-2-fluoropropyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (10) and (*S*)-1-(2-Bromo-3-fluoropropyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (11) (10:1 mixture of 10 and 11). (*S*)-1-Allyl-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**9**) (440 mg, 1.21 mmol) was converted to a mixture of two regioisomers (**10** and **11**) using Olah's reagent (0.31 mL, 1.33 mmol) and crystallized NBS (236 mg, 1.33 mmol), as described in the general procedure, and stirred for 14 h. The crude product was purified by column chromatography (ethyl acetate: cyclohexane 1:5) to yield a mixture of two regioisomers as deep-orange colored gummy solid. Yield: 293 mg (52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.54−1.90 (m, 4H), 3.01−3.10 (m, 1H), 3.27−3.61 (m, 5H), 3.30 (s, 3H), 3.52 (dd, 1H, $^2J_{Hb,Ha}$ = 9.6 Hz, $^3J_{Hb,H}$ = 3.6 Hz), 3.63−3.72 (m, 1H), 3.80−4.20 (m, 2H), 7.11−7.22 (m, 1H), 7.94−8.05 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.2, 29.3, 36.6 (d, $^2J_{C,F}$ = 16.7 Hz), 40.2, 49.8, 59.5, 59.7, 75.2, 90.0 (d, $^1J_{C,F}$ =

190.4 Hz), 109.2, 117.9, 124.9, 137.9, 134.6, 153.8, 157.0, 180.5. ^{19}F NMR (282 MHz, $CDCl_3$): δ −180.5 (m, 1F, $^2J_{Hc,F}$ = 45.3 Hz, $^3J_{Ha+Hb,F}$ = 18.5 Hz, $^3J_{Hd+He,F}$ = 22.8 Hz), −215.2 (m, 1F, $^2J_{Hd+He,F}$ = 47.8 Hz, $^3J_{Hc,F}$ = 19.1 Hz). HRMS (ESI-MicroTof): *m/e* 499.0320 (M + Na)$^+$, calcd for $C_{18}H_{22}BrFN_2NaO_5S$ 499.0314. HPLC t_R = 26.68 min (97.3%).

(*S*)-1-(4-Bromo-3-fluorobutyl)-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (13). (*S*)-1-(But-3-enyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**12**) (100 mg, 0.2 mmol) was converted to a mixture of two regioisomers (**13** and **14**) using Olah's reagent (0.067 mL, 0.29 mmol) and crystallized NBS (51.7 mg, 0.29 mmol), as described in the general procedure, and stirred for 12 h. The crude product was purified by column chromatography (ethyl acetate:cyclohexane 1:1) to yield pure **13** together with its mixture with the other regioisomer **14** as deep-orange colored gummy solids. Yield (as mixture): 102 mg (81%). Spectral data of the secondary fluoride (**13**) is explained below. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.65−1.73 (m, 2H), 1.87−1.95 (m, 2H), 2.15−2.27 (m, 2H), 3.12−3.18 (m, 1H), 3.36 (s, 3H), 3.37−3.47 (m, 2H), 3.51−3.60 (m, 3H), 3.75−3.77 (m, 1H), 3.97 (t, 2H, $^3J_{H,Ha+Hb}$ = 6.9 Hz), 4.67−4.88 (m, 1H), 7.10 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.08 (d, 1H, $^4J_{H,H}$ = 2.1 Hz), 8.13 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$=1.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 31.3 (d, $^2J_{C,F}$ = 20.7 Hz), 32.5 (d, $^2J_{C,F}$ = 25.0 Hz), 36.8 (d, $^3J_{C,F}$ = 3.6 Hz), 49.4, 59.1, 59.2, 74.8, 89.3 (d, $^1J_{C,F}$ = 176.0 Hz), 110.3, 117.4, 124.7, 134.3, 137.6, 153.1, 157.9, 181.5. ^{19}F NMR (282 MHz, $CDCl_3$): δ −179.2 to −179.7 (m, 1F). HRMS (ESI-MicroTof): *m/e* 499.0320 (M + Na)$^+$, calcd for $C_{18}H_{22}BrFN_2NaO_5S$ 499.0314. HPLC t_R = 27.62 min (99.7%).

(*S*)-1-(11-Bromo-10-fluoroundecyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (16) and (*S*)-1-(10-Bromo-11-fluoroundecyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (17) (11:1 mixture of 16+17). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-(undec-10-enyl)isatin (**15**) (100 mg, 0.21 mmol) was converted to a mixture of two regioisomers (**16** and **17**) using Olah's reagent (0.058 mL, 0.25 mmol) and crystallized NBS (43 mg, 0.24 mmol), as described in the general procedure, and stirred for 12 h. The crude product was purified by column chromatography (ethyl acetate:cyclohexane 3.5:6.5) to yield a mixture of two regioisomers as deep-orange colored gummy solid. Yield: 105 mg (87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26−1.61 (m, 18H), 1.69−1.95 (m, 2H), 3.09−3.15 (m, 1H), 3.34−3.52 (m, 4H), 3.36 (s, 3H), 3.60 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.75−3.79 (m, 3H), 4.48−4.73 (m, 1H), 7.04 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.03 (d, 1H, $^4J_{H,H}$ = 1.6 Hz), 8.09 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 24.7 (d, $^3J_{C,F}$ = 4.2 Hz), 26.8, 27.2, 28.8, 29.0, 29.1, 29.2, 29.3, 33.3 (d, $^2J_{C,F}$ = 20.5 Hz), 33.9 (d, $^2J_{C,F}$ = 25.3 Hz), 40.7, 49.4, 59.1, 59.2, 74.8, 92.1 (d, $^1J_{C,F}$ = 174.6 Hz), 110.4, 117.3, 124.5, 133.6, 137.5, 153.7, 157.7, 182.2. ^{19}F NMR (282 MHz, $CDCl_3$): δ −177.6 to −178.1 (m, 1F, $^2J_{H,F}$ = 66.6 Hz, $^3J_{H,F}$ = 19.9 Hz, $^3J_{H,F}$ = 27.7 Hz), −209.5 to −209.9 (dt, 1F, $^2J_{H,F}$ = 47.1 Hz, $^3J_{H,F}$ = 13.9 Hz). HRMS (ESI-MicroTof): *m/e* 597.1430 (M + Na)$^+$, calcd for $C_{25}H_{36}BrFN_2NaO_5S$ 597.1405. HPLC t_R = 36.50 min (99.8%).

(*S*)-1-[4-(2-Bromo-1-fluoroethyl)benzyl]-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (19). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-(4-vinylbenzyl)isatin (**18**) (72 mg, 0.163 mmol) was converted to **19** using Olah's reagent (0.045 mL, 0.196 mmol) and crystallized NBS (33.4 mg, 0.188 mmol), as described in the general procedure, and stirred for 3 h. The crude product was purified by column chromatography (ethyl acetate:cyclohexane 1:1) to yield a deep-orange colored gummy solid. Yield: 60 mg (68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.64−1.72 (m, 2H), 1.85−1.93 (m, 2H), 3.06−3.14 (m, 1H), 3.32−3.44 (m, 2H), 3.33 (s, 3H), 3.53−3.75 (m, 4H), 4.99 (s, 2H), 5.53−5.72 (ddd, 1H, $^2J_{Ha,F}$ = 46.6 Hz, $^3J_{Ha,Hb}$ = 7.1 Hz, $^3J_{Ha,Hc}$ = 4.7 Hz), 6.90 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 7.30 (d, 2H, $^3J_{H,H}$ = 8.2 Hz), 7.41 (d, 2H, $^3J_{H,H}$ = 8.2 Hz), 7.99 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz), 8.05 (d, 1H, $^4J_{H,H}$ = 1.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 33.9 (d, $^2J_{C,F}$ = 28.4 Hz), 44.1, 49.3, 59.1, 59.2, 74.8, 92.1 (d, $^1J_{C,F}$ = 178.5 Hz), 111.1, 117.5, 124.6, 126.7 (d, 2C, $^3J_{C,F}$ = 6.7 Hz), 127.9, 134.2, 134.9 (d, $^5J_{C,F}$ = 1.6 Hz), 137.4, 137.6 (d, $^2J_{C,F}$ = 20.6 Hz), 153.1, 157.8, 181.6. ^{19}F NMR (282 MHz, $CDCl_3$): δ −174.3 to −174.9 (m, 1F, $^2J_{Ha,F}$ = 46.4 Hz, $^3J_{Hb,F}$ = 16.4 Hz, $^3J_{Hc,F}$ = 23.1 Hz, $^4J_{H,F}$ = 6.0 Hz). HRMS (ESI-MicroTof): *m/e* 563.0443 (M + Na)$^+$, calcd for $C_{23}H_{24}BrFN_2NaO_5S$ 563.0447. HPLC t_R = 30.43 min (99.1%).

(*S*)-1-[4-(3-Bromo-2-fluoropropoxy)benzyl]-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (21) and (*S*)-1-[4-(2-Bromo-3-fluoropropoxy)benzyl]-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-isatin (22) (7:3 mixture of 21+22). (*S*)-1-[4-(Allyloxy)benzyl]-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (**20**) (80 mg, 0.17 mmol) was converted to a mixture of two regioisomers (**21** and **22**) using Olah's reagent (0.045 mL, 0.2 mmol) and crystallized NBS (34.8 mg, 0.195 mmol), as described in the general procedure, and stirred for 6 h. The crude product was purified by flash column chromatography (ethyl acetate:cyclohexane 3.5:6.5) to yield a mixture of two regioisomers as deep-orange colored gummy solid. Yield (as mixture): 60 mg (62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65−1.68 (m, 2H), 1.85−1.92 (m, 2H), 3.07−3.16 (m, 1H), 3.33−3.43 (m, 2H), 3.34 (s, 3H), 3.57 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.58−3.75 (m, 3H), 4.22−4.38 (m, 2H, $^2J_{H,F}$ = 19.8 Hz, $^3J_{H,H}$ = 4.2 Hz), 4.68−5.07 (m, 1H), 4.92 (s, 2H), 6.90−6.94 (m, 3H), 7.29 (d, 2H, $^3J_{H,H}$ = 8.5 Hz), 7.98−8.07 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 28.8, 29.4 (d, $^2J_{C,F}$ = 25.9 Hz), 43.9, 49.3, 59.1, 59.2, 67.5 (d, $^2J_{C,F}$ = 27.4 Hz), 74.8, 111.2, 115.3, 117.5, 124.5, 126.7, 129.2, 134.0, 137.4, 153.3, 157.8, 158.2, 181.9. ^{19}F NMR (282 MHz, $CDCl_3$): δ −183.4 to −183.8 (m, 1F, $^2J_{Hc,F}$ = 55.6 Hz, $^3J_{H,F}$ = 16.1 Hz, $^3J_{H,F}$ = 19.4 Hz, $^3J_{H,F}$ = 26.6 Hz), −216.3 to −216.8 (m, 1F, $^2J_{Hd,F}$ = 46.7 Hz, $^2J_{He,F}$ = 52.1 Hz, $^3J_{Hc,F}$ = 15.6 Hz). HRMS (ESI-MicroTof): *m/e* 623.0827 (M + Na + CH_3OH)$^+$, calcd for $C_{25}H_{30}BrFN_2NaO_7S$ 623.0833. HPLC t_R = 31.20 min (95.1%).

General Procedure for the Synthesis of *N*-(Epoxyalkyl) Isatins. To a solution of (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) in 3 mL of dry DMF,2.5 equiv of anhydrous potassium carbonate was added under argon atmosphere and the reaction mixture was stirred for 30 min at ambient temperature. Then the reaction mixture was cooled to 0 °C and an excess of the alkylating agent (2−3 equiv) was slowly added. The reaction mixture was stirred for 30 min at 0 °C and for a further 12−14 h in the case of simple alkyl halides and for 6−8 h in the case of benzylic chlorides and (*S*)-(+)-glycidyl nosylate (in the synthesis of **29**). The reaction mixture was diluted with 20 mL of ethyl acetate and, after 15 min of stirring, was filtered. Removal of the solvents in vacuo furnished the crude product, which was purified by silica gel chromatography.

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[(*S*)-oxiran-2-ylmethyl]isatin (28). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) (500 mg, 1.54 mmol) was converted to **28** using anhydrous K_2CO_3 (532 mg, 3.85 mmol) and (*S*)-(+)-glycidyl nosylate (800 mg, 3.06 mmol) as described in the general procedure and stirred for 6 h. The crude product was purified by column chromatography (ethyl acetate:toluene 8:2) to yield a deep-orange colored solid. Yield: 328 mg (56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.64−1.72 (m, 2H), 1.87−1.92 (m, 2H), 2.73 (dd, 1H, $^2J_{Hd,He}$ = 4.4 Hz, $^3J_{Hd,Hc}$ = 2.6 Hz), 2.93 (t, 1H, $J_{He,Hc+Hd}$ = 4.2 Hz), 3.10−3.16 (m, 1H), 3.22−3.25 (m, 1H), 3.36 (s, 3H), 3.37−3.46 (m, 2H), 3.49 (dd, 1H, $^2J_{Ha,Hb}$ = 15.2 Hz, $^3J_{Ha,Hc}$ = 6.6 Hz), 3.60 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.74−3.78 (m, 1H), 4.50 (dd, 1H, $^2J_{Hb,Ha}$ = 15.2 Hz, $^3J_{Hb,Hc}$ = 2.1 Hz), 7.29 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.06 (d, 1H, $^4J_{H,H}$ = 1.7 Hz), 8.10 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 28.8, 42.9, 44.8, 49.4, 49.8, 59.1, 59.2, 74.9, 111.9, 117.3, 124.5, 134.2, 137.6, 153.6, 157.9, 181.5. HRMS (ESI-MicroTof): *m/e* 403.0942 (M + Na)$^+$, calcd for $C_{17}H_{20}N_2NaO_6S$ 403.0934. HPLC t_R = 20.50 min (100%).

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[2-(oxiran-2-yl)ethyl]isatin (30). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) (400 mg, 1.23 mmol) was converted to **30** using anhydrous K_2CO_3 (425 mg, 3.08 mmol) and **23** (559 mg, 3.7 mmol), as described in the general procedure, and stirred for 12 h. The crude product was purified by column chromatography (ethyl acetate:toluene 9:1) to yield a deep-orange colored gummy solid.

Yield: 301 mg (62%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.53−1.74 (m, 3H), 1.87−1.94 (m, 2H), 2.18−2.25 (m, 1H, $^2J_{Hb,Ha}$ = 14.4 Hz, $^3J_{Hb,H}$ = 7.3 Hz, $^3J_{Hb,Hc}$ = 3.4 Hz), 2.52−2.54 (ddd, 1H, $^2J_{Hd,He}$ = 4.7 Hz, $^3J_{Hd,Hc}$ = 2.6 Hz, $^4J_{Hd,H}$ = 1.2 Hz), 2.80 (dd, 1H, $^2J_{He,Hd}$ = 4.8 Hz, $^3J_{He,Hc}$ = 4.1 Hz), 3.01 (m, 1H, $^3J_{Hc,He}$ = 3.6 Hz, $^3J_{Hc,Ha}$ = 6.5 Hz, $^3J_{Hc,Hb}$ = 10.5 Hz), 3.12−3.18 (m, 1H), 3.36 (s, 3H), 3.36−3.39 (m, 1H), 3.41−3.45 (m, 1H), 3.59 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.74−3.78 (m, 1H), 3.91−4.00 (m, 2H), 7.10 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.06 (d, 1H, $^4J_{H,H}$ = 1.8 Hz), 8.10 (dd, 1H,$^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.1, 28.8, 30.5, 37.8, 47.0, 49.3, 49.6, 59.1, 59.2, 74.8, 110.3, 117.3, 124.6, 134.0, 137.5, 153.3, 157.9, 181.7. HRMS (ESI-MicroTof): *m/e* 417.1094 $(M + Na)^+$, calcd for $C_{18}H_{22}N_2NaO_6S$ 417.1091. HPLC t_R = 20.93 min (96.8%).

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[9-(oxiran-2-yl)nonyl]isatin (33). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) (200 mg, 0.61 mmol) was converted to **33** using anhydrous K_2CO_3 (212 mg, 1.54 mmol) and **24** (382 mg, 1.54 mmol), as described in the general procedure, and stirred for 12 h. The crude product was purified by column chromatography (ethyl acetate:toluene 4.5:5.5 to yield a deep-orange colored gummy solid. Yield: 260 mg (86%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.30−1.57 (m, 14H), 1.66−1.74 (m, 4H), 1.89−1.94 (m, 2H), 2.47 (dd, 1H, $^2J_{Hd,He}$ = 5.1 Hz, $^3J_{Hd,Hc}$ = 2.7 Hz), 2.75 (t, 1H, $J_{He,Hd+Hc}$ = 4.4 Hz), 2.88−2.99 (m, 1H), 3.12−3.17 (m, 1H), 3.36 (s, 3H), 3.36−3.39 (m, 1H), 3.42−3.46 (m, 1H), 3.60 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.63−3.78 (m, 3H), 7.02 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.05 (d, 1H, $^4J_{H,H}$ = 1.8 Hz), 8.10 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.1, 25.9, 26.8, 27.2, 28.8, 29.1, 29.2, 29.3, 29.4, 32.4, 40.6, 47.1, 49.3, 52.3, 59.1, 59.2, 74.8, 110.3, 117.3, 124.5, 133.7, 137.4, 153.7, 157.7, 182.1. HRMS (ESI-MicroTof): *m/e* 515.2198 $(M + Na)^+$, calcd for $C_{25}H_{36}N_2NaO_6S$ 515.2186. HPLC t_R = 33.62 min (95.5%).

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[4-(oxiran-2-yl)benzyl]isatin (36). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) (100 mg, 0.3 mmol) was converted to **36** using anhydrous K_2CO_3 (106 mg, 0.77 mmol) and **25** (156 mg, 0.925 mmol), as described in the general procedure, and stirred for 8 h. The crude product was purified by column chromatography (ethyl acetate:toluene 1:1) to yield a deep-orange colored gummy solid. Yield: 116 mg (84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.62−1.70 (m, 2H), 1.84−1.93 (m, 2H), 2.79 (dd, 1H, $^2J_{Hb,Hc}$ = 5.4 Hz, $^3J_{Hb,Ha}$ = 2.5 Hz), 3.07−3.13 (m, 1H), 3.16 (dd, 1H, $^2J_{Hc,Hb}$ = 5.4 Hz, $^3J_{Hc,Ha}$ = 4.1 Hz), 3.33−3.43 (m, 2H), 3.34 (s, 3H), 3.56 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.69−3.74 (m, 1H), 3.85 (dd, 1H, $^3J_{Ha,Hb}$ = 2.6 Hz, $^3J_{Ha,Hc}$ = 3.9 Hz), 4.96 (s, 2H), 6.89 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 7.28−7.34 (m, 4H), 7.98 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz), 8.05 (d, 1H, $^4J_{H,H}$ = 1.7 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.1, 28.8, 44.2, 49.3, 51.3, 51.9, 59.1, 59.2, 74.8, 111.2, 117.5, 124.6, 126.5, 127.7, 133.7, 134.2, 137.4, 138.3, 153.2, 157.8, 181.7. HRMS (ESI-MicroTof): *m/e* 511.1522 $(M + Na + CH_3OH)^+$, calcd for $C_{24}H_{28}N_2NaO_7S$ 511.1509. Because of the instability of the product under acidic conditions in polar solvents (TFA/MeCN/H_2O), purity could not be established by HPLC.

(*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[4-(oxiran-2-ylmethoxy)benzyl]isatin (38) (3:1 mixture of 2 diastereomers). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]isatin (**1**) (150 mg, 0.46 mmol) was converted to **38** using anhydrous K_2CO_3 (160 mg, 1.15 mmol) and **27** (183 mg, 0.925 mmol) as described in the general procedure and stirred for 8 h. The crude product was purified by column chromatography (ethyl acetate:toluene 5.5:4.5) to yield a deep golden-yellow colored gummy solid. Yield: 187 mg (83%).

Major Diastereomer. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.65−1.69 (m, 2H), 1.85−1.91 (m, 2H), 2.75 (dd, 1H, $^2J_{Hd,He}$ = 4.9 Hz, $^3J_{Hd,Hc}$ = 2.6 Hz), 2.90−2.92 (m, 1H, $^2J_{He,Hd}$ = 4.9 Hz, $^3J_{He,Hc}$ = 4.2 Hz), 3.08−3.12 (m, 1H), 3.33 (s, 3H), 3.33−3.37 (m, 2H), 3.39−3.42 (m, 1H), 3.57 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.70−3.73 (m, 1H), 3.92 (dd, 1H, $^2J_{Ha,Hb}$ = 11.1 Hz, $^3J_{Ha,Hc}$ = 5.8 Hz), 4.25 (dd, 1H, $^2J_{Hb,Ha}$ = 11.0 Hz, $^3J_{Hb,Hc}$ = 3.0 Hz), 4.90 (s, 2H), 6.90−6.93 (m, 3H), 7.25−7.30 (m, 2H), 7.98 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz), 8.04 (d, 1H, $^4J_{H,H}$ = 1.9 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.0, 28.8, 43.9, 44.6, 49.3, 50.0, 59.0, 59.1, 68.8, 74.8, 111.2, 115.3, 117.4, 124.4, 126.2, 129.1, 134.0, 137.3, 153.3, 157.8, 158.6, 181.9.

Minor Diastereomer. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.65−1.69 (m, 2H), 1.85−1.91 (m, 2H), 2.77 (dd, 1H, $^2J_{Hd,He}$ = 4.9 Hz, $^3J_{Hd,Hc}$ = 2.6 Hz), 2.90−2.92 (m, 1H, $^2J_{He,Hd}$ = 4.9 Hz, $^3J_{He,Hc}$ = 4.2 Hz), 3.08−3.12 (m, 1H), 3.33−3.37 (m, 2H), 3.33 (s, 3H), 3.39−3.42 (m, 1H), 3.57 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.70−3.73 (m, 1H), 3.96 (dd, 1H, $^2J_{Ha,Hb}$ = 11.0 Hz, $^3J_{Ha,Hc}$ = 5.7 Hz), 4.24 (dd, 1H, $^2J_{Hb,Ha}$ = 11.1 Hz, $^3J_{Hb,Hc}$ = 3.2 Hz), 4.62 (s, 2H), 6.90−6.93 (m, 3H), 7.25−7.30 (m, 2H), 7.98 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz), 8.04 (d, 1H, $^4J_{H,H}$ = 1.9 Hz). ^{13}C NMR (150 MHz, $CDCl_3$): δ 24.0, 28.8, 43.9, 44.7, 49.3, 50.1, 59.0, 59.1, 68.7, 74.8, 111.2, 114.7, 117.4, 124.4, 126.2, 128.6, 134.0, 137.3, 153.3, 157.8, 158.6, 181.9. HRMS (ESI-MicroTof): *m/e* 541.1616 $(M + Na + CH_3OH)^+$, calcd for $C_{25}H_{30}N_2NaO_8S$ 541.1615. HPLC t_R = 27.30 min (99.2%).

General Procedure for the Synthesis of *N*-(Fluorohydroxyalkyl) Isatins (Procedure A for the synthesis of 29 and 34+35). To a dried, argon flushed Teflon vessel with a rubber septum, Olah's reagent (2−5 equiv) in dry methylene chloride (2 mL) was cooled to 0 °C. Using a syringe, a solution of terminal epoxide **28** or **33** in 5 mL of dry methylene chloride was injected dropwise along with gentle stirring. After the addition, the reaction mixture was stirred at 0 °C for 30 min and then at RT for 12−16 h. The reaction mixture was then cooled to 0 °C, quenched with chilled saturated sodium bicarbonate solution (20 mL), and the aqueous layer extracted completely with excess CH_2Cl_2. All the organic extracts were sequentially washed with 1 N HCl solution (15 mL), followed by 5% sodium bicarbonate solution (15 mL), dried over $MgSO_4$, and filtered. The solvent was then removed in vacuo to afford the crude product, which was purified by silica gel chromatography.

General Procedure for the Synthesis of *N*-(Fluorohydroxyalkyl) Isatins (Procedure B for the synthesis of 31 + 32, 37, and 39). To a dried, argon flushed round-bottomed flask, terminal epoxide **30**, **36**, or **38** was added followed by $Et_3N \cdot 3HF$ (100−200 equiv), and the reaction mixture was heated at 80−90 °C for 2−16 h (depending on the substrate) under argon atmosphere. The reaction mixture was then cooled to 0 °C, diluted with CH_2Cl_2 (20 mL), and quenched with chilled saturated sodium bicarbonate solution (20 mL). After thoroughly shaking and separating the two layers, the aqueous layer was extracted completely with excess CH_2Cl_2. All the organic extracts were dried over $MgSO_4$ and filtered. The solvent was then removed in vacuo to afford the crude product, which was purified by silica gel chromatography.

(*S*)-1-[(*S*)-3-Fluoro-2-hydroxypropyl]-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (29). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[(*S*)-oxiran-2-ylmethyl]isatin (**28**) (90 mg, 0.236 mmol) was converted to **29** using Olah's reagent (0.27 mL, 1.18 mmol), as described in the general procedure A, and stirred for 14 h. The crude product was purified by column chromatography (ethyl acetate:toluene 8:2) to yield a light-orange colored solid. Yield: 48 mg (51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.62−1.75 (m, 2H), 1.84−1.95 (m, 2H), 3.07−3.15 (m, 1H), 3.32−3.46 (m, 2H), 3.36 (s, 3H), 3.58 (dd, 1H, $^2J_{Hb,Ha}$ = 9.5 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.70−3.77 (m, 1H), 3.86−4.01 (m, 2H), 4.24−4.34 (m, 1H), 4.38−4.44 (ddd, 1H, $^2J_{Hd,F}$ = 43.3 Hz, $^2J_{Hd,He}$ = 15.2 Hz, $^3J_{Hd,Hc}$ = 5.4 Hz), 4.60−4.67 (ddd, 1H, $^2J_{He,F}$ = 46.9 Hz, $^2J_{He,Hd}$ = 13.9 Hz, $^3J_{He,Hc}$ = 4.1 Hz), 7.25 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.01 (d, 1H, $^4J_{H,H}$ = 1.7 Hz), 8.07 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 42.9 (d, $^3J_{C,F}$ = 7.5 Hz), 49.4, 59.1, 59.2, 68.9 (d, $^2J_{C,F}$ = 20.1 Hz), 74.8, 84.3 (d, $^1J_{C,F}$ = 170.7 Hz), 111.7, 117.4, 124.4, 133.9, 137.4, 154.0, 157.8, 181.6. ^{19}F NMR (282 MHz, $CDCl_3$): δ −231.7 (dt, 1F, $^2J_{H,F}$ = 46.9 Hz, $^3J_{H,F}$ = 18.1 Hz). HRMS (ESI-MicroTof): *m/e* 455.1257 $(M + Na + CH_3OH)^+$, calcd for $C_{18}H_{25}FN_2NaO_7S$ 455.1259. HPLC t_R = 18.82 min (98.0%).

(*S*)-1-(4-Fluoro-3-hydroxybutyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (32). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)-sulfonyl]-1-[2-(oxiran-2-yl)ethyl]isatin (**30**) (90 mg, 0.236 mmol) was converted to a mixture of two regioisomers (**31** and **32**) using

$Et_3N\cdot 3HF$ (1.85 mL, 11.4 mmol), as described in the general procedure B, and stirred for 8 h. The crude product was purified by column chromatography (ethyl acetate:toluene 8:2) to yield pure **32**, together with its mixture with the other regioisomer **31** as deep-orange colored gummy solids. Yield (as mixture): 48 mg (71%). Spectral data of the primary fluoride (**32**) is explained below. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.63−1.97 (m, 6H), 2.60 (s, 1H), 3.04−3.17 (m, 1H), 3.33−3.48 (m, 2H), 3.36 (s, 3H), 3.60 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.73−3.76 (m, 1H), 3.89−4.12 (m, 3H), 4.24−4.31 (ddd, 1H, $^2J_{Hd,F}$ = 47.6 Hz, $^2J_{Hd,He}$ = 15.7 Hz, $^3J_{Hd,Hc}$ = 6.3 Hz), 4.44−4.52 (ddd, 1H, $^2J_{He,F}$ = 46.8 Hz, $^2J_{He,Hd}$ = 12.9 Hz, $^3J_{He,Hc}$ = 3.5 Hz), 7.18 (d, 1H, $^3J_{H,H}$ = 8.2 Hz), 8.05 (d, 1H, $^4J_{H,H}$ = 1.6 Hz), 8.11 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 29.5 (d, $^3J_{C,F}$ = 6.1 Hz), 37.2, 49.4, 59.1, 59.2, 67.5 (d, $^2J_{C,F}$ = 19.8 Hz), 74.8, 86.3 (d, $^1J_{C,F}$ = 170.3 Hz), 110.6, 117.4, 124.6, 134.0, 137.6, 153.5, 158.3, 181.8. ^{19}F NMR (282 MHz, $CDCl_3$): δ −228.1 (dt, 1F, $^2J_{Hd+He,F}$ = 46.8 Hz, $^3J_{Hc,F}$ = 17.5 Hz). HRMS (ESI-MicroTof): *m*/*e* 469.1417 (M + Na + $CH_3OH)^+$, calcd for $C_{19}H_{27}FN_2NaO_7S$ 469.1415. HPLC t_R = 19.57 min (97.3%).

(*S*)-1-(10-Fluoro-11-hydroxyundecyl)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (34). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[9-(oxiran-2-yl)nonyl]isatin (**33**) (110 mg, 0.22 mmol) was converted to a mixture of two regioisomers (**34** and **35**) using Olah's reagent (0.1 mL, 0.447 mmol), as described in the general procedure A, and stirred for 10 h. The crude product was purified by column chromatography (ethyl acetate:cyclohexane 6:4) to yield **34** as deep-orange colored gummy solid. Yield: 60 mg (52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30−1.95 (m, 20H), 3.09−3.17 (m, 1H), 3.34−3.48 (m, 2H), 3.36 (s, 3H), 3.60 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.64−3.79 (m, 5H), 4.46−4.68 (m, 1H), 7.02 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.04 (d, 1H, $^4J_{H,H}$ = 1.7 Hz), 8.10 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 24.9 (d, $^3J_{C,F}$ = 4.1 Hz), 26.8, 27.2, 28.8, 29.1, 29.2, 29.3, 29.4, 30.9 (d, $^2J_{C,F}$ = 20.4 Hz), 40.7, 49.4, 59.1, 59.2, 65.1 (d, $^2J_{C,F}$ = 21.8 Hz), 74.8, 94.7 (d, $^1J_{C,F}$ = 167.6 Hz), 110.4, 117.4, 124.6, 133.7, 137.5, 153.7, 157.8, 182.1. ^{19}F NMR (282 MHz, $CDCl_3$): δ −189.4 to −189.9 (m, 1F, $^2J_{Hc,F}$ = 49.9 Hz, $^3J_{H,F}$ = 16.5 Hz, $^3J_{H,F}$ = 22.9 Hz, $^3J_{H,F}$ = 29.3 Hz). HRMS (ESI-MicroTof): *m*/*e* 535.2248 (M + Na)$^+$, calcd for $C_{25}H_{37}FN_2NaO_6S$ 535.2249. HPLC t_R = 30.75 min (100%).

(*S*)-1-[4-(1-Fluoro-2-hydroxyethyl)benzyl]-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (37). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[4-(oxiran-2-yl)benzyl]isatin (**36**) (90 mg, 0.236 mmol) was converted to **37** using $Et_3N\cdot 3HF$ (0.27 mL, 1.18 mmol), as described in the general procedure B, and stirred for 2 h. The crude product was purified by column chromatography (ethyl acetate:toluene 7:3) to yield a deep golden-yellow colored solid. Yield: 48 mg (51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 1H), 1.61−1.72 (m, 2H), 1.83−1.91 (m, 2H), 3.07−3.12 (m, 1H), 3.32−3.44 (m, 2H), 3.34 (s, 3H), 3.57 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.68−3.97 (m, 3H), 4.98 (s, 2H), 5.46−5.66 (ddd, 1H, $^2J_{Ha,F}$ = 48.3 Hz, $^3J_{Ha,Hb}$ = 6.8 Hz, $^3J_{Ha,Hc}$ = 3.6 Hz), 6.90 (d, 1H, $^3J_{H,H}$ = 8.2 Hz), 7.30 (d, 2H, $^3J_{H,H}$ = 8.2 Hz), 7.41 (d, 2H, $^3J_{H,H}$ = 8.2 Hz), 8.00 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz), 8.05 (d, 1H, $^4J_{H,H}$ = 1.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 44.1, 49.3, 59.1, 59.2, 66.3 (d, $^2J_{C,F}$ = 24.5 Hz), 74.8, 92.2 (d, $^1J_{C,F}$ = 172.6 Hz), 111.1, 117.5, 124.6, 126.6 (d, 2C, $^3J_{C,F}$ = 7.1 Hz), 127.8, 134.2, 134.4 (d, $^5J_{C,F}$ = 1.4 Hz), 137.0 (d, $^2J_{C,F}$ = 20.0 Hz), 137.4, 153.1, 157.8, 181.7. ^{19}F NMR (282 MHz, $CDCl_3$): δ −186.5 (ddd, 1F, $^2J_{Ha,F}$ = 48.8 Hz, $^3J_{Hb,F}$ = 19.5 Hz, $^3J_{Hc,F}$ = 28.2 Hz), −187.4 (ddd, 1F, $^2J_{Ha,F}$ = 48.6 Hz, $^3J_{Hb,F}$ = 20.7 Hz, $^3J_{Hc,F}$ = 28.2 Hz). HRMS (ESI-MicroTof): *m*/*e*: 531.1565 (M + Na + $CH_3OH)^+$, calcd for $C_{24}H_{29}FN_2NaO_7S$ 531.1572. HPLC t_R = 23.82 min (97.7%).

(*S*)-1-[4-(3-Fluoro-2-hydroxypropoxy)benzyl]-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-isatin(40). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)sulfonyl]-1-[4-(oxiran-2-ylmethoxy)benzyl]isatin (**38**) (90 mg, 0.236 mmol) was converted to **39** using $Et_3N\cdot 3HF$ (0.27 mL, 1.18 mmol), as described in the general procedure B, and stirred for 16 h. The crude product was purified by column chromatography (ethyl acetate:toluene 7:3) to yield a deep golden-yellow colored gummy solid. Yield: 48 mg (45%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 1H), 1.57−1.71 (m, 2H), 1.82−1.93 (m, 2H), 3.05−3.14 (m, 1H), 3.30−3.45 (m, 2H), 3.34 (s, 3H), 3.58 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.8 Hz), 3.68−3.75 (m, 1H), 4.06 (d, 2H, $^3J_{Ha+Hb,Hc}$ = 4.9 Hz), 4.20−4.30 (m, 1H), 4.60−4.67 (ddd, 1H, $^2J_{Hd,F}$ = 47.1 Hz, $^2J_{Hd,He}$ = 12.7 Hz, $^3J_{Hd,Hc}$ = 3.0 Hz), 4.63−4.70 (ddd, 1H, $^2J_{He,F}$ = 47.1 Hz, $^2J_{He,Hd}$ = 14.1 Hz, $^3J_{He,Hc}$ = 4.6 Hz), 4.91 (s, 2H), 6.91 (d, 2H, $^3J_{H,H}$ = 8.6 Hz), 6.93 (d, 1H, $^3J_{H,H}$ = 8.2 Hz), 7.29 (d, 2H, $^3J_{H,H}$ = 9.9 Hz), 8.00 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.8 Hz), 8.05 (d, 1H, $^4J_{H,H}$ = 1.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 43.9, 49.4, 59.1, 59.2, 67.9 (d, $^3J_{C,F}$ = 6.8 Hz), 68.9 (d, $^2J_{C,F}$ = 20.3 Hz), 74.8, 83.5 (d, $^1J_{C,F}$ = 169.7 Hz), 111.2, 115.2, 117.5, 124.5, 126.5, 129.2, 134.0, 137.4, 153.3, 157.8, 158.4, 181.9.^{19}F NMR (282 MHz, $CDCl_3$): δ −232.8 (dt, 1F, $^2J_{Hd+He,F}$ = 47.0 Hz, $^3J_{Hc,F}$ = 18.5 Hz). HRMS (ESI-MicroTof): *m*/*e* 561.1676 (M + Na + $CH_3OH)^+$, calcd for $C_{25}H_{31}FN_2NaO_8S$ 561.1677. HPLC t_R = 25.32 min (98.3%).

(*S*)-1-[(*S*)-2,3-Dihydroxypropyl]-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]isatin (40). (*S*)-5-[1-(2-Methoxymethylpyrrolidinyl)-sulfonyl]-1-[(*S*)-oxiran-2-ylmethyl]isatin (**28**) (80 mg, 0.21 mmol) was converted to **40** using Olah's reagent (0.48 mL, 2.1 mmol), as described in the general procedure A, except that after stirring the reaction mixture at 0 °C for 20 min, it was quenched with saturated sodium bicarbonate solution and then vigorously stirred at ambient temperature for 2 h. The crude product was purified by column chromatography (5% MeOH/CH_2Cl_2) to afford a yellow colored gummy solid. Yield: 43 mg (51%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.46 (s, 1H), 1.59−1.70 (m, 2H), 1.83−1.94 (m, 2H), 2.05 (s, 1H), 3.06−−3.14 (m, 1H), 3.30−3.46 (m, 2H), 3.36 (s, 3H), 3.58 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.66−3.74 (m, 1H), 3.85−4.01 (m, 2H), 4.24−4.64 (m, 3H), 7.26 (d, 1H, $^3J_{H,H}$ = 8.1 Hz), 7.99−8.06 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 43.0, 49.3, 59.1, 59.2, 68.7, 69.0, 74.8, 111.7, 117.4, 124.3, 133.8, 137.4, 154.1, 158.6, 181.7. HRMS (ESI-MicroTof): *m*/*e* 421.1034 (M + Na)$^+$, calcd for $C_{17}H_{22}N_2NaO_7S$ 421.1040. HPLC t_R = 17.68 min (99.8%).

(*S*)-1-(3,4-Dihydroxybutyl)-5-[1-(2-methoxymethylpyrrolidinyl)-sulfonyl]isatin (41). A crude mixture of (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-1-[2-(oxiran-2-yl)ethyl]isatin (**30**) (50 mg, 0.126 mmol) was converted to **41** by absorbing it on silica gel and then loading it on a silica gel column packed in CH_2Cl_2:MeOH (95:5) as the solvent system. The mass was then allowed to remain on the column for 8 h and then eluted with CH_2Cl_2:MeOH (95:5) to afford a yellow colored gummy solid. Yield: 29 mg (55%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.25 (s, 1H), 1.65−1.95 (m, 6H), 2.27−2.32 (m, 1H), 3.09−3.17 (m, 1H), 3.35−3.53 (m, 3H), 3.37 (s, 3H), 3.60 (dd, 1H, $^2J_{Hb,Ha}$ = 9.4 Hz, $^3J_{Hb,H}$ = 3.9 Hz), 3.65−3.77 (m, 3H), 3.84−3.93 (m, 1H), 4.02−4.12 (m, 1H), 7.19 (d, 1H, $^3J_{H,H}$ = 8.3 Hz), 8.05 (d, 1H, $^4J_{H,H}$ = 1.7 Hz), 8.11 (dd, 1H, $^3J_{H,H}$ = 8.3 Hz, $^4J_{H,H}$ = 1.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.1, 28.8, 30.4, 37.4, 49.4, 59.1, 59.2, 66.4, 68.9, 74.8, 110.7, 117.4, 124.6, 134.0, 137.6, 153.5, 158.4, 181.8. HRMS (ESI-MicroTof): *m*/*e* 467.1462 (M + Na + $CH_3OH)^+$, calcd for $C_{19}H_{28}N_2NaO_8S$ 467.1459. HPLC t_R = 15.70 min (96.4%).

Radiosynthesis of [^{18}F]Triethylamine trihydrofluoride ([^{18}F]$Et_3N\cdot 3HF$). For the preparation of 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG), no-carrier-added aqueous [^{18}F]fluoride ions were produced on an RDS 111e cyclotron (CTI-Siemens) by irradiation of a 1.2 mL water target using 10 MeV proton beams on 97.0% enriched [^{18}O]water by the ^{18}O(p,n)^{18}F nuclear reaction. After discharging the cyclotron target, it was rinsed with water (1.2 mL) for injection and this rinsed batch of aqueous [^{18}F]fluoride ions was also used for the preparation of [^{18}F]$Et_3N\cdot 3HF$. This batch was transferred to a polypropylene (PP) tube, and the water was carefully distilled off at 120 °C in vacuo. Then, $Et_3N\cdot 3HF$ (2.0 μL, 2.0 mg, 12.5 μmol) and acetonitrile (10 μL) were added and the mixture was heated to 60 °C for 35 min in an ultrasound bath. The solution was used for the next step without further purification.

Radiosynthesis of (*S*)-1-[4-(1-[^{18}F]Fluoro-2-hydroxyethyl)benzyl]-5-[1-(2-methoxymethyl-pyrrolidinyl)sulfonyl]isatin [^{18}F]37. The [^{18}F]$Et_3N \cdot 3HF$ solution was transferred to a PP tube containing (*S*)-5-[1-(2-methoxymethylpyrrolidinyl)sulfonyl]-1-[4-(oxiran-2-yl)-benzyl]isatin (**36**) (6.5 mg, 14.2 μmol). The tube was sealed, and the mixture was stirred for 45 min at 120 °C. The reaction was then quenched by adding 1 mL of acetonitrile and diluted with a 10 mL injection of water. The mixture was passed through a Waters Sep-Pak Light C18 cartridge. The cartridge was washed with additional 10 mL of water injection and eluted with 0.5 mL hot DMF (warmed to 120 °C before elution). The eluate was diluted with 0.5 mL injection of water and purified by radio-RP-HPLC (flow = 4 mL/min; eluents: A, CH_3CN/H_2O/TFA, 950/50/1; B, CH_3CN/H_2O/TFA, 50/950/1; time program: eluent B from 70% to 10% in 35 min, from 10% to 70% in 5 min). The product fraction of compound [^{18}F]37 (retention time t_R([^{18}F]**37**) = 18.6 min) was evaporated to dryness in vacuo and redissolved in 1 mL of H_2O:EtOH (9:1 v/v). Product compound [^{18}F]**37** was obtained in a radiochemical yield of 7%. Radiochemical purity was >95% and the specific activity was <1 GBq/μmol at the end of synthesis, considering a synthesis time of 220 min. The chemical identity of [^{18}F]**37** was proven by HPLC on the above-mentioned gradient system providing coelution of [^{18}F]**37** and its nonradioactive counterpart **37** that was added to the product fraction beforehand.

In Vitro Enzyme Inhibition Assays (Table 1). The binding potencies of compounds **1**−**22** and **28**−**41** were assayed for recombinant human caspases-1, -3, -6, and -7 (Alexis Biochemicals (Switzerland)) using their peptide-specific substrates (Alexis Biochemicals (Switzerland)) Ac-YVAD-AMC (Ac-Tyr-Val-Ala-Asp-AMC, caspase-1), Ac-DEVD-AMC (Ac-Asp-Glu-Val-Asp-AMC, caspase-3), Ac-VEID-AMC (Ac-Val-Glu-Ile-Asp-AMC, caspase-6), and Ac-DEVD-AMC (Ac-Asp-Glu-Val-Asp-AMC, caspase-7) as already described.[30,31] The enzymatic activity of the caspases was determined by measuring the accumulation of the cleaved fluorogenic product AMC (7-amino-4-methylcoumarin). Reaction rates showing inhibitory activity of the nonradioactive model inhibitor were measured with a Fusion universal microplate analyzer (PerkinElmer) at excitation and emission wavelengths of 360 and 460 nm, respectively. All assays were performed at a volume of 200 μL at 37 °C in reaction buffer.[30,27] Buffers contained the nonradioactive compounds **1**−**22** and **28**−**41** in DMSO in single doses (end concentrations 500 μM, 50 μM, 5 μM, 500 nM, 50 nM, 5 nM, 500 pM, 50 pM, or 5 pM). Recombinant caspases were diluted into the appropriate buffer to a concentration of 0.5 units per assay (= 500 pmol substrate conversion after 60 min). After 10 min incubation time, the peptide substrates (end concentration 10 μM) were added and reacted for further 10 min. The IC_{50} values were determined by nonlinear regression analysis using the *XMGRACE* program (Linux software).

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Supporting Information Available: Experimental procedures and spectroscopic data of starting materials and assignment of spectroscopic data of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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